RECENT ADVANCES IN DIAGNOSTIC APPROACHES FOR PLANT PATHOGEN DETECTION

Abstract

Emerging plant pathogens persist as a serious threat to the food security and stability of ecosystems, casting a looming shadow over the global economy. Plant pathogens afflicting one of the biggest threats to agriculture worldwide, capable of amplifying harvest losses by as much as 30 percent of the total global annual economic output, resulting in the depletion of food production worth billions of dollars. Therefore, it becomes important to precisely delineate the issue and explore solutions for addressing these ailments. Traditional methods are deemed insufficient in terms of precision, accuracy and sensitivity required for the identification of plant pathogens. Traditional diagnostic techniques involve culture-dependent morphological approaches that are cumbersome, time-intensive and become even more formidable when confronted with biotrophic pathogens. To bridge the existing gap, researchers have developed specific, sensitive and effective molecular techniques as a diagnostic tool for plant diseases, holding the potential to enhance decision-making in disease management, addressing many limitations of conventional methods. Molecular strategies offer significant advancements in crop diagnostics, enabling the early identification and containment of quarantine pathogens, as well as facilitating more comprehensive integrated tools for disease control. A wide variety of molecular tools are progressively gaining importance as indispensable assets across various facets of plant pathogen diagnosis. These include enzyme immunoassays,

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lateral flow assays, polymerase chain reaction, loop-mediated isothermal amplification, helicase-dependent amplification, rolling circle amplification, and next-generation sequencing. Characterized by enhanced sensitivity and specificity, reduced time requirements, and transformative capabilities, these methods stand to revolutionize the approach that farmers and pathologists adopt in diagnosing crop diseases. The future for molecular approaches in point-of-care diagnostics appears exceedingly promising, especially when coupled with uncomplicated DNA extraction procedures, reagent stabilization methodologies, and seamless integration into portable devices.

Keywords: Detection, disease, molecular approach, next-generation sequencing

I. INTRODUCTION

The global population is continuously growing; the primary objective of modern agriculture is the sustainable rise in crop production that keeps pace with the increase in the number of mouths to feed. As agricultural endeavors expand rapidly nourishing the planet's increasing population, the impact of crop diseases and pests are on food production and quality has become a significant concern. These afflictions are capable of amplifying harvest losses by as much as 30 percent of the total global annual economic output, resulting in the depletion of food production worth billions of dollars (Rizzo *et al*., 2021). Hence, it becomes important to precisely delineate the issue and explore solutions for addressing these ailments. The precision in the diagnosis of disease and detection of pathogens hold paramount importance since lacking this ability would restrain our comprehension and in many cases our ability to manage the diseases. Detection entails identifying the presence of a specific target organism within a sample, even in asymptomatic individuals. On the other hand, diagnosis is concerned with recognizing the nature and origin of the disease issue, focusing on plants showing symptoms. The capacity to detect a disease in its early stages proves pivotal for farmers to avert disease outbreaks and adopt precise control measures. For instance, unexpected weather conditions can profoundly impact crops, influencing both host susceptibility and pathogen aggression, occasionally culminating in severe disease outbreaks, compromising crop productivity, and inflicting financial losses to farmers (Savary *et al*., 2017). Conventional techniques for identifying plant pathogens primarily depend on descriptive approaches relying on the interpretation of visual symptoms as well as isolation, culture and laboratory-based identification, encompassing; physiological, biochemical and pathogenicity tests done on pathogens (Lau and Botella, 2016). The precision and reliability of these methods largely hinge on the expertise and proficiency of the person performing them. Diagnosis necessitating culture, can prove time intensive and impractical when quick outcomes are required, by culturing the sample on specific media and awaiting for the potential pathogens to develop under controlled conditions, this procedure takes days or even weeks and even more challenging in case of biotrophic pathogens (Donoso and Valenzuela, 2018). Indeed, the identification of a number of plant pathogens poses a significant challenge often necessitating a deep understanding of taxonomy. Timely disease management decisions are complicated by all these factors. Traditional methods are deemed insufficient in terms of precision, accuracy and sensitivity required for the identification of plant pathogens. In order to close the current gap, researchers are actively developing strong molecular techniques, including diagnostic tools and kits customized for plant diseases. These technologies have the potential to improve disease management decision-making processes and overcome the constraints of traditional testing procedures. A suite of molecular approaches has been developed to detect infections with greater sensitivity, specificity, and speed, reducing the arduous and time-consuming nature of the operation. The development of precise diagnostic tests is critical in the accurate diagnosis and ongoing monitoring of plant pests and diseases. Concurrently, the diagnostic products developed as a result of these efforts permit diligent plant health surveillance. Misidentification and incorrect diagnosis have serious consequences, jeopardizing disease control efforts and resulting in the wastage of time and resources. Hence precise and rapid disease diagnosis along with early identification in disease management, is critical for efficiently avoiding pest and pathogen establishment and dissemination, hence minimizing subsequent consequences (Piombo *et al*., 2021). Molecular diagnostic methods have quickly advanced in recent decades, ushering in a new age in agricultural diagnostic technology. These advances have aided in the development

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of effective optional instruments for identifying plant diseases. Polymerase chain reaction (PCR) types such as conventional PCR, real-time PCR, reverse transcription (RT)-PCR, nested PCR, and multiplex PCR are examples of molecular methodologies (Capote *et al.*, 2012). Immunological methods such as enzyme-linked immunosorbent assay (ELISA), plate-trapped antigen-ELISA, and double antibody sandwich-ELISA (Uehara-Ichiki *et al.*, 2013), as well as lateral flow assays (LFA) (De Boer and Lopez, 2012), are critical. Furthermore, loop-mediated isothermal amplification, helicase-dependent amplification, rolling circle amplification, and recombinase polymerase amplification are examples of isothermal methodologies (Lau and Botella, 2016), whereas DNA barcoding (Choudhary *et al.,* 2021) and Next-generation sequencing (NGS) (Chalupowicz *et al.*, 2019) are examples of innovative dimensions. This study concisely summarises the influential molecular methods used in the detection of plant pathogens and their potential importance in revealing plant diseases.

II. IMMUNOLOGICAL DIAGNOSTIC METHOD

This method is based on a special reaction of antigen-antibodies.

1. **Enzyme-Linked Immunosorbent Assay (ELISA):** ELISA is a solid-phase test designed for the detection of plant viruses (Clark and Adams 1977). It employs enzyme-labeled antibodies that react with a substrate to produce a color change, allowing a specific chemical in a sample to be identified and quantified (Uehara-Ichiki *et al*., 2013). This approach is commonly used in plant pathology as a diagnostic tool since it is relatively sensitive and specific, allowing for reliable diagnosis. An enzyme is coupled to particular antibodies against a specific pathogen in this process, and a compound known as a "conjugate" is formed. The conjugate contains sap from the diseased plant. The enzyme-labeled antibodies react with the pathogen antigen. The enzyme conjugated to the antibody is deactivated upon antigen adsorption, but the enzyme inside non-reacting antibodies remains active. The enzyme-influenced substrate is then added. Colorimetry, or the measurement of product color change, is used to determine the degree of enzyme participation in the reaction. The presence and concentration of the pathogen are determined by comparing the drop in enzyme activity across experimental and control settings (Khasanov, 2011). The enzyme immunoassay test is based on the interaction of two different reactions: immunochemical and enzymatic. An antibody develops a bond with a pathogen antigen during the immunochemical process. The enzymatic reaction, on the other hand, involves the modification of a material by an immunological reaction catalyzed by an enzyme. The sample's color changes as a result of this enzymatic action. The substrate is the material manipulated by the enzyme, whereas the product of the enzymatic reaction is the subsequent substance formed by enzyme activity. According to Khakimov *et al*. (2022), chemically connected (conjugated) antibodies include horseradish peroxidase, beta-galactosidase, and alkaline phosphatase. Direct, indirect, and sandwich ELISA are a few of the several solid-phase ELISA procedures. A targeted antibody that has been enzyme-conjugated is used in direct ELISA. In indirect ELISA, a secondary generic antibody, such as enzyme-conjugated anti-rabbit or anti-mouse, is used to identify a particular antibody. Through two layers of antibodies—capture and detection antibodies—Sandwich ELISA measures antigens. The procedure for sandwich ELISA may be summed up as follows; a) the target antigen from the test sample is captured by a particular antibody that covers the micro-titre plate. b) The detection antibody is added,

and it binds to the antigen that was caught. c) A secondary antibody that is enzyme-linked is added and adheres to the detecting antibody. d) The substrate is introduced and is then transformed by enzymes into a detectable form (Khakimov *et al*., 2022). While invasive methods like ELISA are useful for diagnosing viral infections, Sakudo *et al*. (2006) found that they may not be the best option in terms of cost-efficiency, speed, and precision. For instance, Wang *et al*. (2012) demonstrated high reliability, sensitivity, and disease specificity by successfully using a rabbit anti-Southern rice black stripe disease virus (SRBSDV) antiserum to diagnose SRBSDV infection in rice plants. Furthermore, Uehara-Ichiki *et al*. (2013) demonstrated the usefulness and simplicity of the application of the double antibody sandwich enzyme immunoassay (DAS-ELISA) approach for routine diagnostic detection of RSV in insect vectors. The early summer, before the rice cultivation phase, is the critical time for proactive monitoring of virulent insect vectors in order to predict the occurrence of rice streaks, a disease with severe negative effects. This method, which uses plastic multi sticks to process up to 96 insect samples at once, is distinguished by its simplicity. Furthermore, in a Chinese setting, the accurate application of PCR-ELISA and Dot-ELISA methodologies allowed for the precise detection of Wheat dwarf virus (WDV) within crude extracts of WDV-infected wheat plant tissues (Zhang *et al*., 2018). Using the ELISA method as part of their serological tests, Klap *et al.* (2020) demonstrated the identification of *Tobamovirus* ToBRFV and *Potexvirus* PepMV, which cause symptoms resembling those of the mottled yellow spot virus in tomatoes in Israel. In addition, the *Apple mosaic virus, Citrus tristeza virus*, and *Fusarium circinatum* have all been identified (Nabi *et al*., 2020; Raeisi *et al*., 2022; Mkhize and Mangena, 2021). In spite of its impressive ability to detect pathogens, ELISA has a number of disadvantages. The testing procedure can take up to 24 hours, and compared to many other molecular methods, it is noticeably less sensitive (Stackhouse *et al*., 2020). Due to the complexity of the technique and the high cost of cell culture media required for producing particular antibodies, ELISA implementation can also be expensive and labor-intensive (Sakamoto *et al*., 2018). While polyclonal antibodies are effective at detecting pathogens, their specificity can occasionally be lacking. On the other hand, monoclonal antibodies provide greater specificity but come at a higher price. Thanks to ELISA's increased sensitivity to viruses, the development of antibodies against plant viruses has achieved remarkable success; however, when used to detect more complex organisms, such as fungal plant pathogens, its efficacy is significantly reduced (McCartney *et al*., 2003). ELISA is ineffective in this situation due to the decreased efficacy that also affects the detection of bacteria (Fang and Ramasamy, 2015). As a result, ELISA is ineffective for locating the bacterial and fungal pathogens that cause diseases related to rice. Additionally, the inherent lack of specificity in ELISA-used antibodies can result in cross-reactivity with a wide range of strains exhibiting different symptoms, making accurate differentiation difficult (Scala *et al.,* 2018).

2. **Lateral Flow Immunoassay:** The Lateral Flow Assay (LFA) is an immunochemical diagnostic method that promotes antigen-antibody interactions. It is based on the principles of thin-layer chromatography. Koczula described this interaction in test strips or cassettes specifically for this purpose in 2016. Notably, these tests are carried out inside small, disposable cartridges, which eliminates the need for challenging pipetting or washing procedures and makes them possible for even inexperienced people. The elimination of a cold chain need is a noteworthy benefit. Secondary antibodies, frequently connected to gold or silver nanoparticles, are essential to this procedure. In as little as 10

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to 30 minutes, LFA technology makes it possible to detect pathogen-specific antigens and/or antibodies. Notably, LFAs are among the first disease surveillance strategies that are suitable for livestock keepers (De Boer and Lopez, 2012). The architecture of the strip is remarkable, consisting of a membrane and functional pads, such as a sample pad, a conjugate pad, and an absorbent pad. Specific capture probes, such as antibodies or nucleic acids, are immobilized within the nitrocellulose membranes to generate the test and control lines. A nitrocellulose membrane, a sample pad, and an additional adjuvant pad are the typical components of lateral flow test strips. The nitrocellulose membrane stands out as one of the crucial elements of lateral flow assays (LFAs), acting as a crucial platform for assay response and detection, as emphasized by Koczula in 2016. A modest sample volume is put to the chosen sample pad during the testing process. To facilitate, a buffer solution has been used to pre-treat this pad. As described by Donoso and Valenzuela in 2018, commercial LFA devices are being used to identify a variety of plant pathogens including *Phytophthora* spp., *Ralstonia solanacearum*, and Potato virus Y. The lateral flow immunoassay is a diagnostic technique that efficiently evaluates both the quantity and quality of a target chemical in complex combinations. In this procedure, a sample is applied to the test strip, and the results are quickly apparent within 5 to 10 minutes. According to Boisen *et al.* in 2015, the usage of these testing tools has increased because of their affordability, user-friendliness, and ability to produce quick results. Given that the success of lateral flow assays (LFA) depends on the tagging and binding affinity of the biomolecules under investigation, the sensitivity and specificity of this method present considerable obstacles. LFA has other drawbacks as well, most notably a limited capacity to simultaneously detect several target analytes. A high concentration of molecules is necessary to provide favorable results. Consequently, LFA might produce false negative findings when analyzing samples that only contain a small number of target molecules.

III. NUCLEIC ACID-BASED

Finding the pathogen's systematic location is of utmost importance when diagnosing plant diseases. The identification of the species composition of the pathogen is essential for the creation of efficient control measures. Numerous nucleic acid techniques are used to identify pathogenic organisms (viruses, phytoplasmas, bacteria, fungi, etc.) within plants, including polymerase chain reaction (PCR) and its various iterations (Dyakov and Elansky, 2019).

1. **Polymerase Chain Reaction:** Kary Mullis, an American biochemist who won the Nobel Prize for this discovery in 1993, invented the polymerase chain reaction (PCR) in 1983. Specific DNA segment must be amplified under controlled circumstances with the help of enzymes in PCR. If the nucleotide sequence in the DNA matrix meets certain requirements, millions of copies of the target fragment are produced under these circumstances. According to Pililshikova (2013), conventional PCR typically amplifies fragments up to 3000 base pairs. In 1991, Rasmussen and Wolf used PCR for the first time to identify the bacterium *Pseudomonas syringae* pv *pisi* in the context of diagnosing plant diseases. Since then, PCR has gained widespread acceptance as a result of its superior performance to conventional diagnostic methods for plant disease prevention (Balodi *et al.*, 2017). Specificity, sensitivity, efficiency, rapidity, and universality are characteristics of PCR-based diagnostics (Henson and French, 1993)

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2. Types of PCR:

- **Conventional Polymerase Chain Reaction (PCR):** Conventional PCR, also known as endpoint PCR, was created by Kary Mullis in the middle of the 1980s and is regarded as a titanic achievement in the field of molecular biology (Mullis and Faloona, 1987). Beyond the constraints of conventional methodologies, this ground-breaking method has revolutionized the precise identification of plant pathogens. The success of PCR in the characterization of plant pathogens is made possible by ongoing technological advancements. The DNA template, Taq polymerase, two primers, deoxynucleotide triphosphates (dNTPs), buffer solution, and the presence of divalent cations (Mg2+ or Mn2+) in addition to monovalent cations (K+) are all required for the PCR procedure (Azizi and Lau, 2022). PCR is frequently used to detect plant pathogens because it is skilled at amplifying small amounts of DNA and frequently facilitating visualization through gel electrophoresis analysis. Notably, target DNA variability and nucleic acid extraction techniques have an impact on the sensitivity of PCR (Bastien *et al*., 2008). The ability of PCR to amplify small amounts of DNA makes it particularly useful for identifying pathogens with long latency periods between infection and symptom manifestation. However, conventional PCR has some drawbacks, such as a lack of robustness and susceptibility to contamination (Boonham *et al*., 2008). When compared to conventional pathogen detection methods, its improved specificity and sensitivity offset these drawbacks. Notably, due to the limitations of agarose gel electrophoresis in resolving yield disparities, its resolution is somewhat hampered, roughly tenfold. Despite these limitations, PCR is still a valuable tool for identifying plant pathogens. Traditional PCR continues to be one of the most widely used methods for identifying plant pathogens. As an illustration, *Pantoea ananatis* and *P. stewartii* subspecies *indologenes* were identified by Azizi *et al*. (2019) as the causal agents of bacterial blight disease (BLB) in rice in Malaysia. Similar to this, as mentioned by Shweta *et al*. (2018), PCR has been useful in southern Karnataka (India) in characterizing *Ralstonia solanacearum* isolates accountable for bacterial wilt in tomatoes. Additionally, *Fusarium oxysporum* f.sp. l*ycopersici* (Fol) strains isolated from diseased tomatoes were successfully identified by Murugan *et al.* (2020) using the PCR method. However, there are some restrictions with the PCR method. A significant worry is the increased risk of contamination, which could lead to the dispersing of amplicons into the environment (Hajia, 2018).

- **Nested PCR:** Nested PCR, a variation of the polymerase chain reaction, amplifies a DNA fragment using two sets of PCR primers. The first set of primers produces a fragment similar to how PCR normally works. The second set of primers, referred to as nested primers, subsequently attach to the first PCR product to allow for the amplification of a second DNA fragment that is shorter than the original product. This method has found widespread use in the characterization and identification of various fungi (Qin *et al*., 2011). Nested PCR was used to detect the presence of orchid disease caused by *Phytophthora* species. Using the nested PCR approach, this technique successfully identified two different Phytophthora species, namely *P. palmivora* and *P. parasitica*, as the causal agents (Tsai *et al*., 2006). The fact that this specific PCR method requires the execution of two amplification cycles in separate tubes raises the risk of contamination, it should be noted (Rahman *et al*., 2013).

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- **Multiplex PCR:** By simultaneously using various primer pairs within a single reaction to simultaneously detect various targeted DNA sequences, multiplex PCR offers an effective way to save time and money. When plants are battling multiple pathogens, this method becomes especially important in the field of plant pathology. Specifically, target pathogenic fungi-related fragments are simultaneously amplified and identified, and their molecular sizes are determined on agarose gels (Aslam *et al*., 2017). Given that multiple pathogens typically infect plants, the accuracy of DNA synthesis is significantly influenced by amplicon size, making this approach extremely valuable for identifying plant pathogens. As an example, the multiplex PCR technique was used to simultaneously identify the fungi that cause disease, such as *F. oxysporum, B. cactivora, P. nicotinae,* and *P. cactorum* responsible for infecting inoculated cacti with severe diseases (Ji-Cho *et al*., 2016).

- **Reverse Transcription-PCR:** Since its inception, traditional PCR has undergone numerous improvements, which have significantly expanded its applications and diagnostic capabilities across numerous biological and medical domains (Tang *et al*., 1997). While some PCR variants initially had trouble distinguishing between viable and non-viable organisms, reverse transcription PCR (RT-PCR) marked a significant advancement by successfully overcoming this limitation. This discovery is related to the mRNA degradation process that occurs in dead cells. Thus, as demonstrated by Capote *et al*. in 2012, RT-PCR (Reverse Transcription Polymerase Chain Reaction) can now detect the presence of mRNA, thereby confirming cellular viability. This method starts by converting RNA into complementary DNA (cDNA) using random primers and an RT enzyme and then amplifies the result using a PCR-based strategy. RT-PCR is most often used to identify and treat infections brought on by RNA-containing pathogens, particularly retroviruses. The development and evaluation of vaccine effectiveness and antimicrobial therapies both benefit greatly from the ability to identify RNA-containing viruses. Notably, the *Fusarium graminearum* fungi that cause Fusarium ear disease (FEB), which affects cereals like wheat, rye, barley, oats, and corn, have been quantified using RT-PCR.

- **Real-Time PCR:** The most effective method for locating plant pathogens is PCR. Real-time PCR has been developed in particular to precisely track changes in mRNA levels. This is especially important when dealing with circumstances in which there are only modest amounts of available cells or tissues. The development of real-time PCR, also known as quantitative PCR (qPCR), dates back to Kary Mullis's landmark work in the 1980s, which represented a significant improvement and a revolutionary evolution of conventional PCR techniques (Mullis, 1990). Real-time operations heat cycler used in PCR may subject individual samples to particular light wavelengths. Oligonucleotide probes that generate fluorescence as amplification develops are used to identify amplicons (Azizi and Lau, 2022). The PCR procedure requires several cycles, and the quantity of amplified DNA/RNA contained in the sample is directly related to the strong light released during amplification. To detect and diagnose Dickeya fangzhongdat-induced bleeding cancer illness in pears in China, researchers used TaqMan real-time PCR testing based on the elongation factor G (fusA) gene (Tian *et al*., 2020). The *Tomato leaf curl new delhi virus* (ToLCNDV) was effectively detected using this strategy, as Luigi et al. convincingly proved in 2020. A similar real-time, quick, highly sensitive, and straightforward DNA-based polymerase chain

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reaction (RT-PCR) test was developed by He *et al.* in 2020. This technique made it possible to identify cultivars of tea plants that were resistant and to distinguish between diseases from various *Colletotrichum* species based on their level of aggression. This technique has taken the place of the less accurate and sensitive practise of measuring the size of lesions at infection locations on leaves (He *et al*., 2020). However qPCR's reliance on pre-existing sequence data for the particular target gene is a noteworthy drawback. As a result, only recognized genes may be used using qPCR (Smith and Osborn, 2009).

3. Isothermal Amplification:

- **Loop-Mediated Isothermal Amplification (LAMP):** Due to its speed, simplicity, usability, and affordable equipment needs, LAMP has emerged as a highly successful contemporary technique that is becoming increasingly popular as a viable alternative to PCR. Loop-Mediated Isothermal Amplification, or LAMP, is a technique for amplifying specific DNA sequences. Its main objective was to detect the hepatitis B virus (HBV) by achieving DNA amplification in an isothermal environment when it was first developed by Notomi *et al.* in 2000. This approach selectively targets six different portions of the HBV viral DNA using a special set of four primers. These primers consist of two internal primers (FIP and BIP) and two exterior primers (a forward external primer, F3, and a reverse external primer, B3). They include both internal and external components. The advantages that this novel technology has by nature form its basis. The interaction of these four main primers is key to the LAMP strategy. Notably, there are two external primers (FIP and BIP), which include a forward external primer (F3) and a reverse external primer (B3), and two internal primers (FIP and BIP). The autocyclic strand and the single-strand displacement activity of the DNA strand, which has a loop structure, are the two main components of the amplification process. The self-initiating strand and the activity of single-strand displacement inside a DNA strand with a loop structure are both necessary for the amplification process. This technique lasts 45 to 60 minutes at a steady temperature that is normally maintained between 60°C and 65°C (Lafar *et al*., 2020). The creation of starting material, cyclic amplification with elongation, and a recycling phase are the three main phases of this amplification trip (Hardinge and Murray, 2019). With three different ways for amplicon detection, including the use of a turbidimeter, colorimetric detection, agarose gel electrophoresis, or a real-time fluorometric platform, LAMP stands out as a remarkable adaptive technology (Waliulla *et al*., 2020). It's crucial to be aware of the disadvantages of this strategy even if it has many benefits. Because LAMP is particularly prone to the generation of primer dimers, it must be implemented with a thermal block to maintain the temperature at 65 °C (Rani *et al*., 2019). The LAMP test has established its place in the field of plant pathology as a reliable method for identifying several plant diseases. The use of genus- and species-specific PCR primers for *Colletotrichum* and C. *Nymphaae* produced more accurate results in separating this fungus from others within both pure cultures and disease testing, according to a noteworthy recent study by Karimi *et al*. (2020). This differentiation was made possible by the use of the LAMP approach, which exhibits more sensitivity and specificity than traditional PCR. Additionally, the LAMP test efficiently and without the use of laboratory methods detected *Pseudomonas syringae* pv. *tomato* (Pst) in naturally and experimentally infected tomato leaf and stem tissues

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in the field (Chen *et al*., 2020). Notably, in the context of identifying *Tomato brown rugose fruit virus* (ToBRFV), the sensitivity of the LAMP test has shown comparability to qPCR and even outperformed RT-PCR by up to 100 times (Sarkes *et al*., 2020). It's crucial to understand that the LAMP approach does have certain drawbacks despite its achievements. The danger of cross-contamination is a significant problem due to the LAMP technique's increased sensitivity, especially during the amplification step (Tomlinson and Boonham, 2008). This problem is made worse by the fact that some LAMP variations call for opening reaction tubes in order to identify amplicons. In addition, the very complex design of LAMP assays provides a significant challenge given that PCR just needs two primers. Up to six primers are frequently used in LAMP tests (Tomlinson and Boonham, 2008). The amplification time also affects the length of the LAMP operation. According to François *et al.* (2011), the shortest time for amplification is between 60 to 120 minutes, whereas a negative control needs to display amplification for a longer period of 180 minutes (Dhama et al., 2014). These factors provide insight into the difficulties and optimisation tactics related to the LAMP technique.

- **Helicase-Dependent Amplification (HDA):** A different isothermal method called HDA was created by New England Biolabs in 2004 (Vincent *et al*., 2004). Similar to normal PCR in many ways, this isothermal approach enables primers to bind to their complementary target sequences without the need for heat denaturation to separate double-stranded DNA. To produce single-stranded DNA for primer annealing and primer extension under isothermal circumstances, HDA employs DNA helicase. To stop complementary ssDNA strands from rehybridizing and remodeling dsDNA, the process is supplemented with single-stranded binding protein (SSB) and MutL endonuclease. The HDA method, introduced by Vincent *et al.* in 2004, is capable of producing detectable quantities of PCR amplicons within approximately 60 minutes, ready for downstream analysis. This technique has gained popularity as an isothermal approach due to its straightforward reaction protocol. Although it operates on the same primer pair principle as PCR to amplify target sequences, HDA distinguishes itself by streamlining the process, eliminating the need for multiple temperature cycling steps. Compared to LAMP, another isothermal method, HDA offers notable simplicity. LAMP necessitates the design of four relatively complex long primers, requiring a preliminary thermal denaturation step before amplification at a lower temperature, as outlined by Nagamine *et al.* in 2002. HDA has demonstrated success in detecting pathogenic genomic DNA within complex mixtures, even in highly sensitive human blood samples. However, while HDA's attributes make it a promising candidate for developing uncomplicated point-of-care diagnostic tests, it bears the limitation of requiring intricate optimization to ensure harmonized enzymatic activity between helicase and DNA polymerase. Moreover, the presence of Single-stranded Binding Protein (SSB) and MutL, critical to preventing single-stranded DNA from rehybridizing into double-stranded DNA, could potentially exert a significant impact on the ultimate results, as highlighted in Vincent *et al.*'s work. Moreover, there have been reports indicating that HDA can exhibit inefficiency in amplifying lengthy targets, as pointed out by Guatelli *et al.* in 1990. This limitation may stem from the constrained unwinding speed of the UvrD helicase, which operates at a rate of 20 base pairs per second, with fewer than 100 base pairs processed each second. It's noteworthy that while MutL can enhance the unwinding activity of UvrD, it doesn't

contribute to an increase in the processing rate, as observed in the work of Mechanic *et al*. in 2000. A significant breakthrough emerged with the identification of a thermostable helicase, Tte-UvrD, sourced from *Thermoanaerobacter* t*engcongensis*. This specific helicase, well-suited for amplification at higher temperatures, was found to substantially enhance HDA performance. An et al. (2005) documented this notable advancement

- **Rolling Circle Amplification (RCA):** Rolling circle amplification (RCA), a method first presented by Fire and Xu in 1995, makes use of the idea of isothermal amplification. In RCA, a single primer that has been annealed to a circular DNA template is extended using a DNA polymerase with strand-shifting ability, such as 29 DNA polymerase. As shown by Blanco et al. in 1989, this mechanism of strand shifting enables the freshly generated DNA to displace the previously synthesized DNA, resulting in the release of single-stranded DNA (ssDNA). In combination with strand displacement, this enzymatic expansion of the primer results in an extended single-stranded DNA sequence that is characterized by a repeating pattern that completes the circular template.. Rolling circle amplification (RCA) presents a straightforward and uncomplicated isothermal reaction mechanism. With some additional adjustments, linear DNA can also serve as a suitable template for initiating the RCA process. In this context, a linear single-stranded DNA probe can be strategically designed to create an initial hybridization with the target sequence, forming a loop that is then ligated to generate a circular probe prior to commencing RCA. This technique, known as the padlock probe assay, has been effectively employed in the detection of numerous plant diseases, as evidenced by Tian *et al*. in 2014. The versatility of RCA-tracked padlock probes in terms of high multiplex potential and specificity has propelled its popularity in the multiplex detection of plant pathogens. In comparison to PCR, RCA has been noted for its enhanced specificity and reduced susceptibility to non-specific amplification. RCA has successfully detected various plant pathogens, including *Tomato spotted wilt virus* (Wu *et al*., 2016), *Begomovirus* (Pandey *et al*., 2022), and *Fusarium* spp. (Davari *et al*., 2012). Furthermore, RCA offers the advantage of amplifying up to 0.5 megabases of DNA per probe within an overnight incubation, a feat highlighted by Baner *et al*. in 1998. Additionally, the method generates over 109 copies of each circular sequence within just 90 minutes, as demonstrated by Lizardi *et al*. in 1998. This attribute proves advantageous in hybridization-based readouts, particularly in the identification of repeat sequences, significantly enhancing sensitivity, as discussed by Russell *et al*. in 2014.

- **Recombinase Polymerase Amplification:** According to Piepenburg *et al*.'s research, Recombinase Polymerase Amplification (RPA), a brand-new isothermal amplification technology, was launched in 2006 as a way to target certain DNA regions. RPA functions constantly at a single temperature (usually 37–42°C), much like LAMP technology, making it very useful in the field. Its ability to do away with stages like substantial global matrix melting and other temperature cycling—which are traditionally controlled using a thermal cycler—is one of its major benefits. Its applicability for on-site applications is improved by this feature. Oligonucleotide primers and three key enzymes play crucial roles in isothermal amplification: (i) the recombinase enzyme, (ii) single-stranded DNA-binding proteins, and (iii) the Strand

displacement polymerase, as emphasized by Ahmed et al. The recombinase enzyme forms complexes with primers that actively scan the target sequence, prompting displacement of the template strand. This liberated template strand is then anchored by single-stranded DNA-binding proteins, immobilizing the primer. Subsequently, the strand displacement polymerase triggers the initiation of target sequence amplification. Although relatively few instances of its application for detecting plant pathogens, including fungi, bacteria, and viruses, exist in the literature, these studies have demonstrated RPA's superior performance in terms of sensitivity and time compared to traditional PCR-based diagnostics, with amplification times of 10-15 minutes proving sufficient for detection. Notably, the technology's accessibility is increased by employing straightforward DNA extraction techniques, exemplified in the swift and accessible *Plum pox virus* and *Little cherry virus* 2 diagnostic using high-affinity lateral flow strips to detect terminally labeled RPA amplicons, as illustrated by Mekuria *et al*. in 2014. Numerous pathogens have been successfully detected using RPA, including *Cercospora arachidicola* in peanuts (Lin *et al*., 2022), *Xanthomonas oryzae* in rice (Buddhachat *et al*., 2022), and *Sweet potato feathery mottle virus* and *Sweet potato chlorotic stunt virus* in sweet potatoes (Tang *et al*., 2022). With an impressively low detection limit of just 6.25 femtograms of genomic DNA input and specificity exceeding 95%, RPA showcases remarkable sensitivity. However, similar to other discussed technologies, it does possess a limitation, as it's primarily suited for amplifying small DNA fragments. RPA has also been effectively employed for multiplex detection of plant pathogens by combining it with SERS nanotag technology, as detailed by Lau et al. in 2016. They developed a one-step multiplex detection method grounded in RPA-SERS. Furthermore, field detection using this technique has been demonstrated for pathogens like *Botrytis cinerea*, *Pseudomonas syringae*, and *Fusarium oxysporum* in tomato samples, facilitated by a handheld Raman device following a straightforward sampling protocol.

4. **DNA Barcoding:** DNA barcoding has become a flexible technology with tremendous potential for plant disease diagnosis in the era of genomics. The importance of particular barcodes was closely scrutinized when massively parallel and next-generation sequencing methods rose to prominence. A universal strategy is required for the exact identification and classification of phytopathogens, and this has turned into the principal application for the barcode idea. As described by Choudhary *et al*. in 2021, brief genomic fragments (600 bp) have been used in this setting as DNA barcodes for identification. As explained by Hebert *et al*. in 2003, DNA barcoding was first used to identify animals, but it has now been widely broadened to include a variety of living forms from different strata. As proposed by Ortega *et al*. in 2018, using the DNA barcode approach has the potential to expand into illness diagnosis. Employing the DNA barcode approach has recently demonstrated impressive efficiency when used to identify latent diseases or asymptomatic plants. Chinese Mason (*Plattostoma palustre)* cultures were successfully screened for *Cerospora, Colletotrichum*, and *Fusarium* infections using a combination of ITS, partial-tubulin, and histone H3 markers by Hsieh *et al*. (2020). On the other hand, Schoch *et al*. (2012) carried out a screening and discovered that the internal transcribed spacer (ITS) region is a suitable DNA barcode identifier for fungus. Because of its beneficial characteristics—a conserved core area coupled with a highly variable portion, a small size covering 500–800 base pairs, and worldwide applicability—ITS is chosen as the best barcode marker. One advantage of the ITS1 barcode is that it can distinguish

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Basidiomycetes more precisely than Ascomycetes, whose resolution may be somewhat constrained. Contrarily, Bianchi *et al*. in 2018 drew attention to the fact that ITS2 performs best for Ascomycetes resolution while providing less accuracy for Basidiomycetes. It is noteworthy that different taxa have different ITS region lengths, with Basidiomycota, Zygomycota, and Chytridiomycota having longer ITS sequences than Ascomycota and Glomeromycota. Similar attention is given to oomycetes such as *Phytophthora infestans, Phytophthora palmivora, Phytophthora ramorum, and Plasmopara obducens*. According to Kulik *et al*. in 2020, these oomycetes require proper identification in order to create their respective phylogenies. In pursuit of potential genetic markers suitable as fungal barcodes, the Fungal Tree of Life project undertook a comprehensive search. These candidate regions encompassed various genetic loci: the Internal Transcribed Spacer (ITS) region, as outlined by Kiss in 2012; cytochrome c oxidase (COI) subunit I, highlighted by Robideau *et al*. in 2011; the major nuclear subunit of the ribosome, as explored by Seifert in 2009; nuclear ribosomal small, largest, and second largest subunits of RNA polymerase II, along with elongation factor 1-α, as indicated by Geiser *et al*. in 2004; the small subunit of the mitochondrial ribosomal operon; β-tubulin (BenA), elaborated upon by Samson *et al*. in 2004; actin, as discussed by Roe *et al*. in 2010; chitin synthase, covered by Zeng *et al*. in 2012; calmodulin, per Hong *et al*. in 2008; and heat shock protein 90, as detailed by Zhao *et al*. in 2011. An exciting method to understand plant interactions in a swarm setting was developed by Tremblay *et al*. in 2019. By using metabarcoding to examine pollen pellet samples, they were able to do this. A variety of phytopathogens with agricultural importance were successfully detected by the researchers in their investigation, including *Alternaria* sp., *Colletotrichum* sp., *Fusarium* sp., and *Pythium* sp. Their method focused on particular genomic sections, including the ITS1 intergenic region for oomycetes and fungi, the ATP9-NAD9 region for Phytophthora species, and the ITS2 region for various plant species. Co-occurrence analysis and metabarcoding approaches were both used by Cobo-Diaz *et al*. (2019). Their research focused on identifying *Fusarium* species infections as well as other related microbial communities connected to maize stalks.

5. **Next-Generation Sequencing:** Despite being in use for almost ten years, "next-generation sequencing" (NGS) is still used to refer to sophisticated sequencing techniques that produce data on a genomic scale or even larger and are characterized by high parallelism and remarkable performance. According to Daz-Cruz *et al*. (2019), this relatively new technological development has evolved into a crucial tool for several biological fields, including plant pathology. According to Chalupowicz *et al*. (2019), the development of NGS has ignited a revolution in the sector that has transformed how plant pathogens are found and identified. The development of contemporary methods for detecting and characterizing phytopathogens has been considerably accelerated by the introduction of high-throughput sequencing (HTS) technology, moving the field ahead at a faster rate. High-throughput sequencing (HTS) or next-generation sequencing (NGS) techniques have made it possible to simultaneously sequence a large number of species, allowing for the identification of several organisms in a single sample, as described by Azizi *et al*. (2022). Particularly in the domain of plant viruses, HTS/NGS is emerging as a potential method for analyzing initial samples for quarantine or certification aims. HTS/NGS offers significant benefits, subject to thorough technical and quality control, as mentioned by Azizi *et al.* (2022), while making small alterations to current approaches. A wide range of possible uses for DNA sequencing technologies are available, including

comparative and evolutionary study, the discovery of disease-related genes, improvements in breeding, and molecular cloning. These technologies should ideally combine speed, accuracy, usability, and cost-effectiveness. The landscape of DNA sequencing technology and its uses has significantly changed over the past three decades, igniting the genomic age, which is characterized by an abundance of genetic data. The invention of the Sanger technique, also known as the chain termination method, as described by Sanger *et al*. (1977), marked a turning point. The Sanger technique served as a base upon which other advancements were built, despite its early throughput and accuracy limitations. The early developments of the Sanger method, notwithstanding its throughput and accuracy limitations, formed the direct ancestry of various modern sequencing approaches. Amplified sequencing libraries are required by second-generation sequencing technologies, which adds both expensive and time-consuming features. The use of amplified DNA clones is not necessary with third-generation sequencing techniques, which allow the sequencing of individual molecules. From 2004 to 2014, next-generation sequencing (NGS), also known as massively parallel sequencing, saw significant advancements. With the help of these developments, sequencing technology was transformed and the use of massively parallel analysis was brought into it. This method aggregates millions of short-read sequences into much shorter sequences, reducing time requirements, increasing throughput, and greatly simplifying the genome sequencing process at a significantly lower cost. Massively parallel sequencing, commonly known as next-generation sequencing (NGS), which has made considerable advancements between 2004 and 2014, was essential in bridging gaps and revolutionizing the field of sequencing technologies. Due to this progression, technologies for massively parallel analysis, in which millions of short-read sequences are combined into much shorter sequences, have emerged. As Kanzi *et al*. (2020) eloquently demonstrate, this change produced advantages in terms of time efficiency, increased throughput, and particularly simplified genome sequencing operations, all done at a lower cost. The variety of high-throughput DNA sequencing methods now accessible is referred to as "massively parallel and next-generation DNA sequencing" in this article. As described by Ronholm in 2018, the price of sequencing gigabase nucleic acid pairs has dramatically lowered because of the fast development of NGS technology, falling from $1,000 to an astounding $10. The progressive integration of nucleotides into spatially organized DNA templates is painstakingly monitored by these sequencing approaches. It's crucial to remember that at this point, the differentiating features of various sequencing platforms start to show up.

- **Roche 454 Pyrosequencing:** According to Wheeler *et al*. (2008), the innovative Roche 454 pyrosequencing by synthesis method, which debuted in 2005, signified a substantial leap in next-generation sequencing. Through the combined activity of enzymes like ATP sulfurylase, luciferase, and DNA polymerase, this method exploited a unique sequencing chemistry that depended on the emission of visible light. This light's intensity allowed for the accurate monitoring of the sequencing reaction's development since it was inversely correlated with the number of pyrophosphate molecules produced during the synthesis of a new DNA strand. This invention made it possible to simultaneously analyze several samples in parallel in addition to allowing for the detection of the response at particular light intensities. As a result, the output significantly outperformed that of the traditional Sanger sequencing approach. In 2008, Roche unveiled the upgraded 454 GS FLX Titanium

system, which came with notable upgrades. This device could produce 0.7 gigabases of data in a single 24-hour run and had an astounding accuracy rate of 99.99%. The average read length could exceed 700 base pairs. Despite these improvements, this method had a key flaw in that it had a high error rate while sequencing homopolymeric areas, which are repeated lengths of the same nucleotide. The price of the necessary reagents continued to be a serious issue.

- **Illumina (Solexa) HiSeq and MiSeq:** The Illumina sequencer has adopted sequencing synthesis technology and is using chain terminal nucleotides that are fluorescently labeled and detachable. With this strategy, output is improved while reagent costs are kept to a minimum (Metzker, 2010). The procedure, also known as bridge amplification or cluster creation, makes it easier for compact colonies known as polonies to replicate a clonally enriched template DNA (Shendure and Ji, 2008). With shorter read lengths (around 100 base pairs) and a larger output of sequencing data (600 gigabases) in a single run, the sequencer is more cost-effective. At least eight industrial-grade sequencing devices, including the NextSeq 500, HiSeq Series 2500, 3000, and 4000, as well as the HiSeq X Series Five and Ten, will be available from Illumina. These devices provide a variety of results. The Illumina sequencer has adopted sequencing synthesis technology and is using chain terminal nucleotides that are fluorescently labeled and detachable. With this strategy, output is improved while reagent costs are kept to a minimum (Metzker, 2010). The procedure, also known as bridge amplification or cluster creation, makes it easier for compact colonies known as polonies to replicate a clonally enriched template DNA (Shendure and Ji, 2008). With shorter read lengths (around 100 base pairs) and a larger output of sequencing data (600 gigabases) in a single run, the sequencer is more cost-effective. At least eight industrial-grade sequencing devices, including the NextSeq 500, HiSeq Series 2500, 3000, and 4000, as well as the HiSeq X Series Five and Ten, will be available from Illumina. These machines span a range of output capacities, from medium to high (120 - 1500 gigabases). The same sequencing and polony technique is used by MiSeq, a different small-footprint and portable laboratory sequencer, but with better spin rates and outputs ranging from 0.3 to 15 gigabases (Liu *et al*., 2012). Additionally, Illumina has unveiled a cutting-edge technique called TruSeq technology that uses fake long reads. By addressing the issues raised by complex and repetitive transposable components, this advancement improves the capacity for de novo assembly (McCoy *et al*., 2014). This development in next-generation sequencing was crucial in finding *Calonectria pseudonaviculata*, a new fungal pathogen that causes the Sarcococca disease in ornamental plants. The identification procedure was made easier by the Illumina MiSeq technology, which also revealed that the two host isolates' genomes were 51.4 megabases in size. By pinpointing a unique single nucleotide polymorphism shared exclusively by these isolates, the study confirmed their identity as C. *pseudonaviculata* (Malapi-Wight *et al*., 2016). Stone and pome fruits are damaged both after and before harvest by *Monilinia fructicola*, a disease-causing organism known to cause brown rot. Using a multidimensional and hierarchical de novo method, the M. *fructicola* strain Mfrc123 was mapped. This method required combining data from the third-generation long-read sequencing platforms from Pacific Biosciences (PacBio) and Illumina's short-read Next-Generation Sequencing (NGS) (De Miccolis-Angelini *et al*., 2019).Similar to this, a different hybrid approach was used to read the genome of the fungus *Hemileia*

vastarix, which causes coffee rust. The PacBio RS II and Illumina HiSeq technologies were used in this project, which produced a complete genome for the H. *vastatrix* XXXIII strain with 547 megabases (Porto et al., 2019).

- **Sequencing by Oligonucleotide Ligation and Detection (SOLiD):** In 2008, Applied Biosystems Instruments (ABI) was a leader in the development of systems for sequencing by oligonucleotide ligation and detection. In order to use this method, which is based on the Sequencing of 2 Nucleotides by Ligation (SBL) idea, the probe must first be annealed before being ligated to the template (Sendure and Ji, 2008). The SOLiD 5500 W series has previously been used in a variety of applications, including whole-genome, transcriptome, and exome research (Shendure and Ji, 2008). With the use of fluorescently labelled octamer probes, this sequencer repeatedly engages in cycles of annealing and ligation. Finally, the Exact Call Chemistry is used to cross-verify and interpret these sequences (Guzvic, 2013). The redundant nature of interrogating each base twice lends this method a particular benefit. As each base was enquired twice, this gave the platform a major advantage. However, the main drawbacks were the shorter read lengths (50 - 75 bp), the long execution times, and the need for an expert processing infrastructure.

- **Oxford Nanopore Sequencing:** To interpret the nucleotide sequence of DNA, the Oxford Nanopore sequencing (ONT) method was created. Real-time DNA and RNA sequencing is possible with the MinION, a portable device developed by Oxford Nanopore Technologies (ONT) Ltd. in Didcot, UK. The first use of this portable MinION sequencer made it easier to sequence viral genomes on-site. One may generate about 30 gigabases of DNA sequencing data or 7–12 million reads for RNA analysis with one consumable flow cell. Notably, this technique enables readings that span hundreds of kilobases, which is very lengthy. Under 100 grammes in weight, the gadget is extremely light and can be connected to a computer or laptop using a high-speed USB 3.0 cable. It may also be used in conjunction with the MinIT device to enable real-time analytical capabilities. According to Solares *et al*. (2018), the MinION sequencer is commercially available for less than $1,000 USD. A nanopore is simply a very small opening with an interior diameter of around 1 nanometer that is produced within cells from certain transmembrane proteins. The nanopore is exposed to a voltage, which works on the theory of minute variations in electric current inside a conductive liquid. According to Solares *et al*. (2018), the MinION sequencer is commercially available for less than $1,000 USD. A nanopore is simply a very small opening with an interior diameter of around 1 nanometer that is produced within cells from certain transmembrane proteins. The nanopore is exposed to a voltage, which works on the theory of minute variations in electric current inside a conductive liquid. As a strand of moving nucleotides moves through the pore, this happens. Each DNA nucleotide that passes through the nanopore during this procedure obstructs the passage in a different manner. The amplitude of the electric current is altered in unusual ways depending on how much of this obstruction there is. These variations act as identifying indicators for each unique nucleotide. One may compare the process to threading a piece of DNA through a nanopore. Every DNA nucleotide that passes through the nanopore during this process breaks the route in a unique way. Depending on the degree of blockage, the amount of this obstruction causes various changes in the electric current's intensity. These variations serve as identifying indicators for each

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unique nucleotide. It is possible to draw a comparison between this process and threading a piece of DNA through a nanopore. Since the current change may be directly interpreted, it is possible to determine the DNA sequence by identifying changes in the resulting current that are unique to the traversing base. As an alternative, it is possible to build a nanopore specifically to adjust the current when a certain nucleotide passes through it. Oxford Nanopore is well known for its ability to generate extraordinarily long readings. The prolonged read times stand out as one of its most helpful features among its features (Midha *et al.*, 2019). The read length of the sequencing output matches the length of the DNA fragment that is being examined. Notably, the longest reported read has been more than 200 kilobases (KB). The fragments produced by rival methods, which generally provide 30 gigabytes (GB) with 7–12 million readings, pale in comparison to this astounding length. GridIon, one of the most cutting-edge systems in this field, can generate 150 GB of data, while PromethIon boasts a remarkable output of 7–10 terabytes (TB), with future predictions reaching up to 15 TB. These high-capacity systems, together with the handheld. Along with these large-capacity devices, the portable MinION has already established itself as a tool for DNA sequencing on the go. Oxford Nanopore Technologies has started working on the SmidgION project, an even more compact device. With an average read length of 13 kilobases (kb) and a N50 value of 26 kb, this small device reliably and repeatedly generates read sequence data surpassing 6.5 gigabases (Gb) (Schalamun *et al.*, 2019). Applications of the MinION include identifying harmful bacteria and fungus (Purushotham *et al.*, 2020; Fujiyoshi *et al.*, 2020) in industries including agriculture and forestry. Due to its low cost, quick turnaround time, and availability of user-friendly bioinformatics pipelines, nanopore sequencing has attracted interest in clinical laboratories (Petersen *et al.*, 2019). Limitations: Basic call accuracy compared to other platforms.

IV. CONCLUSION AND FUTURE THRUST

Ecosystems, food security, and the global economy continue to be significantly at risk due to the persistent emergence of new plant pathogens. In order to effectively manage and eradicate diseases, plant quarantine protocols, and disease eradication efforts, timely identification of plant infections is essential. With the aid of contemporary technological advancements, there has been notable progress over the past ten years in improving molecular diagnostic techniques for identifying plant diseases. Consistent innovation in plant disease detection has the potential to improve early diagnosis and make it possible for quarantine-related pathogens to be contained more successfully. These developments will also aid in the creation of all-encompassing management plans to combat such diseases. Plant pathogen detection will likely have regulatory repercussions in the upcoming years, particularly for plant import and export screening procedures. It effectively prevents the spread of plant diseases across regions and countries by ensuring that only plants free of pathogens are permitted to cross international borders. Furthermore, by enabling plant breeders to grow disease-resistant plant varieties, the identification of plant pathogens gives them a significant advantage. Plant pathologists also hope to see the development of more advanced molecular methods for disease detection. These cutting-edge techniques might be practical replacements for current approaches, promoting expansion in both agriculture and the global economy. The development of molecular tools for identifying and characterising plant diseases has been the focus of considerable research, but it's important to recognise the crucial role that farmers

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play in this process. Farmers can actively help to identify diseases that affect their crops early on. The use of field-based testing equipment and easily accessible laboratory services are required for the implementation of approachable pathogen monitoring techniques that are suitable for farmers. These pathogen detection techniques must have qualities like affordability, simplicity, resilience, rapidity, extended shelf life, and precise specificity in order to be efficient and user-friendly. The needs of regulators, exporters, importers, extension agents, and those responding to potential crop-related bioterrorist incidents are all met by these practical methods, which are also helpful to growers and producers. Diagnostic benchmarks like sensitivity, specificity, and error rates must be established in order to verify the effectiveness of novel detection and diagnostic techniques. Cost-effective molecular methodologies should be developed to provide precise insights into the prevalence of pathogens or diseases, encompassing more than just their mere presence or absence. When combined with straight forward DNA extraction techniques, methods for stabilising reagents, and seamless integration into portable apparatus, the potential of molecular strategies becomes especially promising for on-site or in-field diagnostics.

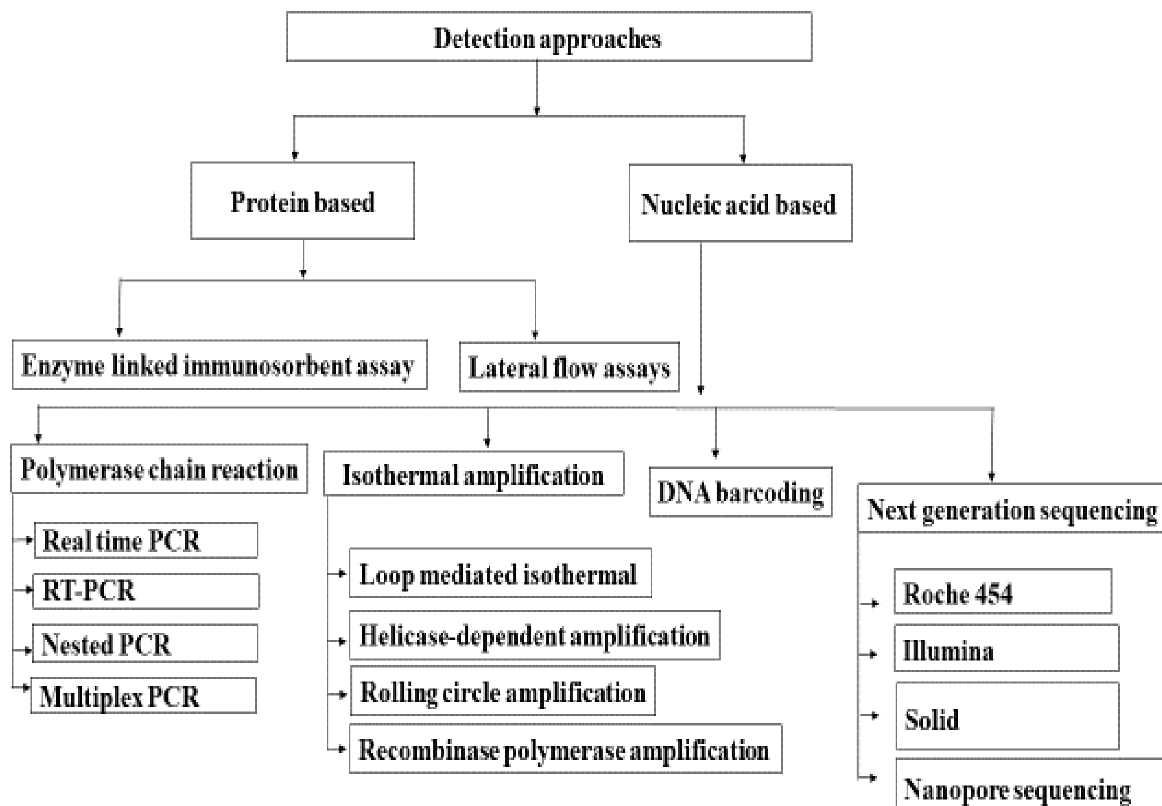

Figure 1: Flow Chart of Detection Techniques

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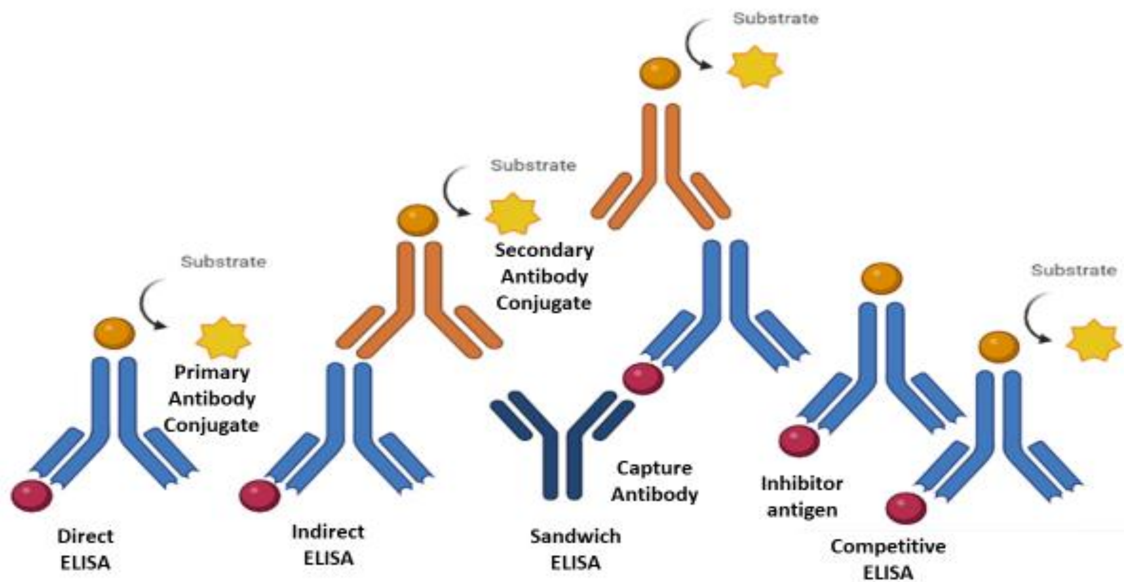

Figure 2: Different Types of ELISA

Table 1: Use of Detection Techniques for Different Pathogens in Crops

Detection Technique	Pathogen	Crop	References
Elisa	*Apple mosaic virus*	Apple	(Nabi *et al.*, 2020)
Elisa	*Citrus tristeza virus*	Citrus	(Raeisi *et al*., 2022)
LFA	*Phytophthora spp., Ralstonia solanacearum, Potato leaf roll virus and Potato virus Y*	Potato	(Koczula, 2016)
qPCR	*Tomato leaf curl New Delhi virus* (ToLCNDV)	Tomato	(Luigi *et al*., 2020)
LAMP	*Xanthomonas citri* pv. *citri*	Citrus	(Webster *et al.,* 2022)
Lamp	*Phytophthora spp*	Strawberry	(Dominika *et al*., 2022)
HDA	*Tomato spotted wilt virus*	Tomato	(Wu *et al*., 2016)
HDA	*Pseudomonas*	Cherry	(Illicic *et al*., 2021)
RCA	*Begomoviruses*	Pulses	(Pandey *et al*., 2022)
RPA	*Xanthomonas oryzae*	Rice	(Buddhachat *et al*., 2022)
DNA Barcoding	*Cercospora, Colletotrichum, and Fusarium*	*Platostoma palustre*	(Hsieh *et al*., 2020)
NGS	*Apple mosaic virus and Apple necrotic mosaic virus*	Apple	(Nabi *et al*., 2022)

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